

THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re : US Patent 5,432,099

Issued: July 11, 1995

To : Roger P. Ekins

Title: DETERMINATION OF AMBIENT  
CONCENTRATION OF SEVERAL  
ANALYTES

*C of C*  
*#29*  
**Certificate**  
**OCT 23 2003**  
**of Correction**

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Suite 2400  
1601 Market Street  
Philadelphia, PA 19103  
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Our File No. 0380 PO0633US1

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**Certificate of Mailing Under 37 C.F.R. §1.8(a):**

I hereby certify that this correspondence is being deposited on October 14, 2003 with the United States Postal Service as first-class mail in an envelope properly addressed to the Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450.

  
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Kimberly St. Clair Kelley

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Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

**SECOND REQUEST UNDER 37 C.F.R. 1.322 FOR**  
**CERTIFICATE OF CORRECTION OF PATENT OFFICE MISTAKE**

Dear Sir:

Submitted herewith in connection with the above identified patent is a Certificate of Correction (PTO form PTO/SB/44) to correct an error which occurred in printing the patent.

The specific error is that in column 14, line 36 (claim 13, line 2), "m<sup>2</sup>" should read --mm<sup>2</sup>--.

As the error was made solely by the Patent and Trademark Office, applicants request expeditious processing, without the patent file, in accordance with the OG notice of September 17, 2002.

In an effort to reduce the overall time required in processing and granting Certificate of Correction requests, the United States Patent and Trademark Office, effective immediately, will expedite processing and granting of patentees' requests where the request is accompanied by paperwork which substantiates that the error incurred is attributable solely to the United States Patent and Trademark Office (i.e., requests filed pursuant to 37 CFR 1.322)...

If such a request for correction was incurred through the fault of the United States Patent and Trademark Office (Office), and is clearly disclosed in the records of the Office, and is accompanied by documentation that unequivocally supports the patentee's assertion(s), a Certificate of Correction will be expeditiously issued. Such supporting documentation can consist of relevant photocopied receipts, manuscript pages, correspondence dated and received by the Office, photocopies of Examiners' responses regarding entry of amendments, or any other validation that supports the patentee's request so that the request can be processed without the patent file.

Accordingly, submitted herewith is a copy of the preliminary amendment filed December 29, 1992 which demonstrates that the error was on the part of the Patent and Trademark Office. Specifically, the preliminary amendment adds new claim 24, which explicitly recites mm<sup>2</sup> (see page -3- of the attached preliminary amendment.) Note that at the time of allowance,

claim 24 was renumbered as claim 13 by the Examiner. Applicants have also attached the date-in stamped post-card receipt from the USPTO as evidence that this preliminary amendment was received by the USPTO.

It is respectfully requested that a Certificate of Correction be issued to correct the above noted error.

In the event that a fee is required, the Commissioner is authorized to charge any payment to the account of the undersigned attorneys, Deposit Account No. 04-1406.

DANN DORFMAN HERRELL and  
SKILLMAN, P.C.

Attorneys for Applicant

By Patrick J. Hagan  
Patrick J. Hagan  
Registration No. 27,643

PJH:ksk

UNITED STATES PATENT AND TRADEMARK OFFICE  
**CERTIFICATE OF CORRECTION**

PATENT NO. : 5,432,099  
DATED : July 11, 1995  
INVENTOR(S) : Roger P. Ekins

Page 1 of 1

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

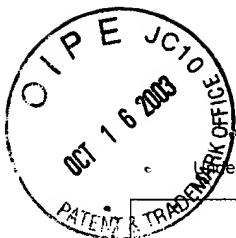
Column 14,  
Line 36, "m<sup>2</sup>" should read -- mm<sup>2</sup> --.

Signed and Sealed this

Twenty-third Day of December, 2003

A handwritten signature in black ink, appearing to read "James E. Rogan", with a long horizontal flourish extending from the bottom of the signature.

JAMES E. ROGAN  
*Director of the United States Patent and Trademark Office*



## UNITED STATES PATENT AND TRADEMARK OFFICE CERTIFICATE OF CORRECTION

PATENT NO. : 5,432,099  
DATED : July 11, 1995  
INVENTOR(S) : Roger P. Ekins

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 14, line 36 in claim 13, line 2 " $m^2$ " should read  $--mm^2--$ .

**MAILING ADDRESS OF SENDER:**

HAGAN, Patrick J.

Dann Dorfman Herrell and Skillman, P.C.

Suite 2400, 1601 Market Street

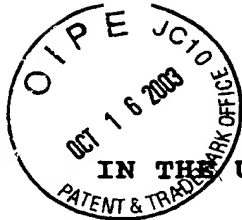
Philadelphia, Pennsylvania 19103

PATENT NO. 5,432,099

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This collection of information is required by 37 CFR 1.322, 1.323, and 1.324. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1.0 hour to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Attention Certificate of Corrections Branch, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of )  
 )  
Roger Philip Ekins )  
 )  
Serial No. 07/984,264 )  
 )  
Filed: December 1, 1992 )  
 )  
For: DETERMINATION OF AMBIENT )  
CONCENTRATION OF SEVERAL )  
ANALYTES )

Certificate of Mailing Under 37 CFR §1.8(a)

I hereby certify that this correspondence is being deposited on December 29, 1992 with the United States Postal Service as first class mail in an envelope addressed to COMMISSIONER OF PATENTS AND TRADEMARKS, Washington, D.C. 20231.

29 December 1992  
Date of Certificate

Patrick J. Hagan  
Patrick J. Hagan  
Attorney for Applicant  
PTO Reg. No. 27,643

PRELIMINARY AMENDMENT

It is respectfully requested that the above-identified application be amended, before issuance of a first Official Action on the merits, as follows.

In the Claims:

Please add the following new claims numbered 12-28.

12. A method for determining the ambient concentrations of a plurality of analytes in a liquid sample of volume V liters, comprising:

loading a plurality of different binding agents, each being capable of reversibly binding an analyte which is or may be present in the liquid sample and is specific for said analyte as compared to the other components of the liquid sample, onto a support means at a plurality of spaced apart small spots such that each spot has a high coating density of one of said binding agents but not more than 0.1 V/K moles of binding agent are present on any spot, where K liters/mole is the equilibrium constant of said binding agent for said analyte;

contacting the loaded support means with the liquid sample to be analyzed, such that each of the spots is contacted in the same step with said liquid sample, the amount of liquid used in said sample being such that only an insignificant proportion of any analyte present in said liquid sample becomes bound to said binding agent specific for said analyte, and

measuring a parameter representative of the fractional occupancy by said analytes of said binding agents at the spots by a competitive or non-competitive assay technique using a site-recognition reagent for each binding agent capable of recognizing either the unfilled binding sites or the filled binding sites on said binding agent, said site-recognition reagent being labelled with a marker enabling the amount of said reagent in the particular location to be measured.

13. A method as claimed in claim 12, wherein each of said spots has a size of less than 1 mm<sup>2</sup>.

14. A method as claimed in claim 13, wherein each of said spots contains more than 10<sup>4</sup> molecules of binding agent.

15. A method as claimed in claim 14, wherein each of said spots has less than 0.01 V/K moles of binding agent.

16. A method as claimed in claim 14, wherein said binding agents used have equilibrium constants for said analytes of from 10<sup>8</sup> to 10<sup>13</sup> liters per mole.

17. A method as claimed in claim 14, wherein said binding agents used have equilibrium constants for said analytes of the order of 10<sup>10</sup> or 10<sup>11</sup> liters per mole.

18. A method as claimed in claim 14, wherein the volume of said liquid sample is not more than 0.1 liter.

19. A method as claimed in claim 14, wherein the volume of said liquid sample is 400 to 1000 microliters.

20. A method as claimed in claim 12, wherein said binding agents loaded onto said support means are antibodies for the analytes whose concentrations are to be determined.

21. A method as claimed in claim 12, wherein said binding agents are labelled with markers enabling the concentration levels of said binding agents to be measured.

22. A method as claimed in claim 21, wherein said binding agents and said site-recognition reagents are labelled with fluorescent markers such that at the individual spots the assay technique for measuring fractional occupancy of the binding agents measures the ratios of the signals emitted by the fluorescent markers.

23. A device for use in determining the ambient concentrations of a plurality of analytes in a liquid sample of volume  $V$  liters, comprising a solid support means having located thereon at high coating density at a plurality of spaced apart small spots a plurality of different binding agents, each binding agent being capable of reversibly binding an analyte which is or may be present in said liquid sample and is specific for said analyte as compared to the other components of said liquid sample, each spot having not more than  $0.1 V/K$  moles of a single binding agent, where  $K$  liters/mole is the equilibrium constant of said single binding agent for reaction with the analyte to which it is specific.

24. A device as claimed in claim 23, wherein each of said spots has a size of less than  $1 \text{ mm}^2$ .

25. A device as claimed in claim 24, wherein each of said spots contains more than  $10^4$  molecules of binding agent.



26. A kit for use in determining the ambient concentration of a plurality of analytes in a liquid sample of volume V litres, comprising:

a solid support means having located thereon at high coating density at a plurality of spaced apart small spots a plurality of different binding agents, each binding agent being capable of reversibly binding an analyte which is or may be present in said liquid sample and is specific for said analyte as compared to the other components of the liquid sample, each spot having not more than  $0.1 V/K$  moles of a single binding agent, where K liters/mole is the equilibrium constant of said single binding agent for reaction with the analyte to which it is specific;

a plurality of standard samples containing known concentrations of the analytes whose concentrations in the liquid sample are to be measured; and

a set of labelled site-recognition reagents for reaction with filled or unfilled binding sites on said binding agents.

27. A kit as claimed in claim 26, wherein each of said spots has a size of less than  $1 \text{ mm}^2$ .

28. A kit as claimed in claim 27, wherein each of said spots contains more than  $10^4$  molecules of binding agent.

Cancel claims 1-11.

Newly added claims 12-28 are fairly based on the disclosure of published International Application No. PCT/GB88/00649 (WO 89/01157) on which the present application is based. See specification at page 14, line 24 through page 5, line 2 and at page 12, lines 2-5.

As reflected in the claims submitted herewith, the invention on which applicant seeks protection is a method for determining ambient concentrations of a plurality of analytes involving, in combination, the use of a small amount of

binding agent on a small spot at high coating density. The combination of these three (3) features imparts surprisingly high sensitivity to the method of the invention. The use of a small amount of binding agent, as proposed in WO 84/01031, which is cited in the International Search Report in the aforesaid WO 89/01157, does not by itself ensure high sensitivity, but rather obviates the requirement of a constant volume sample. Unless a small spot is used, high sensitivity of measurement cannot be achieved because it is impossible to obtain a high signal/noise ratio. The use of a high coating density also contributes substantially to maximizing the signal/noise ratio.

The conventional view before applicant made the present invention was that very small amounts of antibody would pull out only very small amounts of analyte and hence that the signal would be very low and thus the signal/noise ratio would likewise be low. The present invention embodies applicant's surprising discovery, in contravention of the above-noted conventional view, that extremely high sensitivity can be obtained by using a small spot and high coating density.

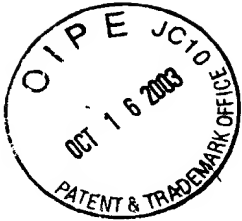
Favorable consideration and allowance of the claims presented herewith is earnestly solicited.

Respectfully submitted,

DANN, DORFMAN, HERRELL AND SKILLMAN  
A Professional Corporation  
Attorneys for Applicant

By Patrick J. Hagan  
Patrick J. Hagan  
Registration No. 27,643

Telephone: (215) 563-4100



In re the Appl<sup>n</sup> of  
Roger Philip Ekins

Serial No. 07/984,264

Filed: 12/1/92

For: DETERMINATION OF  
AMBIENT CONCENTRATION OF  
SEVERAL ANALYTES

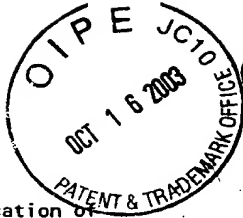
The following paper has been received:  
- Certificate of Mailing  
- Amendment Fee Calculation  
Sheet  
- Preliminary Amendment



Patent and Trademark Office is respectfully requested to place its

STAMP on this POSTAL CARD and place it in the outgoing mail.

Respectfully,  
12/29/92 PJH



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of

Roger P. Ekins

Serial No. 07/984,264

Filed: December 1, 1992

For: DETERMINATION OF AMBIENT CONCENTRATION OF SEVERAL  
ANALYTES

Certificate of Mailing Under 37 CFR §1.8(a)

I hereby certify that this Correspondence is being deposited on December 29, 1992 with the United States Postal Service as first-class mail in an envelope properly addressed to COMMISSIONER OF PATENTS AND TRADEMARKS, Washington, DC 20231.

December 29, 1992  
Date of Certificate

Patrick J. Hagan  
PATRICK J. HAGAN  
Attorney for Applicant(s)  
PTO Registration No. 27,643

Computation of Additional Fee for Amendment

- [ X ] No Additional Fee is required.  
[ ] A check is enclosed in the amount of \$\_\_\_\_\_.

The fee has been calculated as shown below:

CLAIMS AS AMENDED				*SMALL ENTITY		OTHER THAN A SMALL ENTITY	
FOR	CLAIMS AFTER AMDT.	CLAIMS PAID FOR	NUMBER EXTRA	RATE	FEE	RATE	FEE
EFFECTIVE TOTAL CLAIMS	17	- 20	= 0	\$ 10		\$ 20	
IND. CLAIMS	3	- 3	= 0	36		72	
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIMS?				110		220	
PETITION FEE FOR EXTENSION							
				TOTAL		TOTAL	

\*Applicant is a Small Entity, as established by a verified statement filed 2/2/90 in U.S. Serial No. 460,878.

In the event the check is improper, or the fee calculation is in error, the Commissioner is authorized to charge any underpayment or credit any overpayment to the account of the undersigned attorneys, Account No. 04-1406. A duplicate copy of this sheet is enclosed.

DANN, DORFMAN, HERRELL AND SKILLMAN  
A Professional Corporation

By Patrick J. Hagan  
PATRICK J. HAGAN  
PTO Registration No. 27,643

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